

WHAT IS CLAIMED IS:

1. A method for preparing a conjugate vaccine, the method comprising:
reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained;
buffer exchanging the solution of the aldehyde-activated polysaccharide to a pH of from about 7 to about 8;
reacting a protein with hydrazine or adipic acid dihydrazide in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride at a pH of from about 6 to about 7, whereby a solution of an hydrazide-activated protein is obtained;
raising a pH of the solution of the hydrazide-activated protein to from about 7.0 to about 11;
buffer exchanging the solution of the hydrazide-activated protein to a pH of from about 10.0 to about 11.0;
reacting the aldehyde-activated polysaccharide with the hydrazide-activated protein at a pH of from about 6 to about 8, whereby a conjugate comprising one or more C=N double bonds is obtained; and
reducing substantially all of the C=N double bonds of the conjugate to C-N single bonds, whereby a conjugate vaccine capable of stimulating an immune response is obtained.
2. The method according to claim 1, wherein the oxidizing agent comprises NaIO₄.
3. The method according to claim 1, wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged with a HEPES buffer.
4. The method according to claim 1, wherein the solution of the hydrazide-activated protein is buffer exchanged with a Na₂CO₃ buffer.
5. The method according to claim 1, wherein the aldehyde-activated polysaccharide is reacted with the hydrazide-activated protein at a ratio of from about 1:2 to about 2:1.
6. The method according to claim 1, wherein reducing comprises reducing with NaBH₄.
7. The method according to claim 1, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus

polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B Streptococcus polysaccharides.

8. The method according to claim 1, wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.

9. A method for preparing a conjugate vaccine, the method comprising:
reacting a polysaccharide with 1-cyano-4-dimethylammoniumpyridinium tetrafluoroborate, whereby a solution of a cyanate-activated polysaccharide is obtained;

reacting a protein with hydrazine or adipic acid dihydrazide in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride at a pH of from about 6 to about 7, whereby a solution of a hydrazide-activated protein is obtained;

raising the pH of the solution of the hydrazide-activated protein to from about 7.0 to about 11;

buffer exchanging the solution of the hydrazide-activated protein to a pH of from about 10.0 to about 11.0;

reacting the cyanate-activated polysaccharide with the hydrazide-activated protein at a pH of from about 6 to about 8 to yield a conjugate vaccine capable of stimulating an immune response.

10. The method according to claim 9, wherein the step of reacting the cyanate-activated polysaccharide with the hydrazide-activated protein is conducted in the absence of a blocking agent.

11. The method according to claim 9, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B Streptococcus polysaccharides.

12. The method according to claim 9, wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.

13. A method for preparing a conjugate vaccine, the method comprising:
reacting a protein with 1-amino-2,3-propanediol (APDO) in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride at a pH of from about 6 to about 7, whereby a solution of a APDO-modified protein is obtained;

buffer exchanging the solution of the APDO-modified protein to a pH of from about 10.0 to about 11.0;

reacting the APDO-modified protein with an oxidizing agent, whereby a solution of an aldehyde-activated protein is obtained;

buffer exchanging the solution of the aldehyde-activated protein to a pH of from about 10.0 to about 11.0;

reacting a hydrazide-activated polysaccharide with the aldehyde-activated protein at a pH of from about 6 to about 8, whereby a conjugate comprising one or more C=N double bonds is obtained; and

reducing substantially all of the C=N double bonds of the conjugate to C-N single bonds, whereby a conjugate vaccine capable of stimulating an immune response is obtained.

14. The method according to claim 13, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, *Pneumococcus* polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B Streptococcus polysaccharides.

15. The method according to claim 13, wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.

16. The method according to claim 13, wherein the hydrazide-activated polysaccharide is prepared by:

reacting a polysaccharide with an oxidizing agent in a solution, whereby an aldehyde-activated polysaccharide is obtained;

reacting the aldehyde-activated polysaccharide with adipic acid dihydrazide to yield an intermediate comprising one or more C=N double bonds; and

reducing substantially all of the C=N double bonds of the intermediate to C-N single bonds, whereby a hydrazide-activated polysaccharide is obtained.

17. The method according to claim 13, wherein the hydrazide-activated polysaccharide is prepared by:

reacting a polysaccharide with 1-cyano-4-dimethylammoniumpyridinium tetrafluoroborate, whereby a cyanate-functionalized polysaccharide is obtained;

reacting the cyanate-functionalized polysaccharide with adipic acid dihydrazide, whereby a hydrazide-activated polysaccharide is obtained.

18. The method according to claim 13, wherein the hydrazide-activated polysaccharide is prepared by:

reacting a polysaccharide with adipic acid dihydrazide in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride, whereby a hydrazide-activated polysaccharide is obtained.

19. A conjugate vaccine, the conjugate vaccine comprising at least one polysaccharide moiety and at least one protein moiety, wherein the polysaccharide moiety is linked to the protein moiety through at least one linking group of the formula $-C(=O)-NH-NH-CH_2-$.

20. The conjugate vaccine of claim 19, wherein the conjugate vaccine comprises a plurality of polysaccharide moieties and a plurality of protein moieties crosslinked to form a lattice structure by a plurality of linking groups.

21. The conjugate vaccine of claim 19, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B Streptococcus polysaccharides.

22. The conjugate vaccine of claim 19, wherein the protein is selected from the group consisting of tetanus toxoid, diptheria toxoid, CRM₁₉₇, and meningococcal protein.

23. A conjugate vaccine, the conjugate vaccine comprising at least one polysaccharide moiety and at least one protein moiety, wherein the polysaccharide moiety is linked to the protein moiety through at least one linking group of the formula $-C(=O)-NH-NH-C(=NH)-O-$.

24. The conjugate vaccine of claim 23, wherein the conjugate vaccine comprises a plurality of polysaccharide moieties and a plurality of protein moieties crosslinked to form a lattice structure by a plurality of linking groups.

25. The conjugate vaccine of claim 23, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B Streptococcus polysaccharides.

26. The conjugate vaccine of claim 23, wherein the protein is selected from the group consisting of tetanus toxoid, diptheria toxoid, CRM₁₉₇, and meningococcal protein.

27. A conjugate vaccine, the conjugate vaccine comprising at least one polysaccharide moiety and at least one protein moiety, wherein the polysaccharide moiety

is linked to the protein moiety through at least one linking group of the formula $-C(=O)-NH-CH_2-CH_2-NH-NH-$.

28. The conjugate vaccine of claim 27, wherein the conjugate vaccine comprises a plurality of polysaccharide moieties and a plurality of protein moieties crosslinked to form a lattice structure by a plurality of linking groups.

29. The conjugate vaccine of claim 27, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, *Pneumococcus* polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B *Streptococcus* polysaccharides.

30. The conjugate vaccine of claim 27, wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.

31. A conjugate vaccine substantially as herein described with reference to any one of the examples.

32. A method for preparing a conjugate vaccine substantially as herein described with reference to any one of the examples.